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AXONAL CONDUCTION VELOCITY CHANGES FOLLOWING MUSCLE TENOTOMY OR DEAFFERENTATION DURING DEVELOPMENT IN THE RAT

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SUMMARY

1. The conduction velocities of axons supplying the intertransverse caudal muscles of 8-week-old rats were measured. The distribution of conduction velocities was found to be similar to the more commonly studied hind-limb innervation.

2. In animals in which the intertransverse caudal muscles had been tenotomized at birth, however, the conduction velocities attained by both the sensory and the motor nerves by 8 weeks of age were significantly reduced.

3. This effect is limited to growing animals since tenotomy of the intertransverse caudal muscles for the same period in adults had no effect on axonal conduction velocity.

4. Deafferentation of normal intertransverse caudal muscles during development also significantly reduced the conduction velocities attained by the motor innervation, to the same extent as tenotomy had done.

3. These results are discussed in relation to the role of impulse traffic in the development of neuronal dimensions.

INTRODUCTION

When nerve axons grow out into the tissues from the developing C.N.S. their normal growth and function depends on their making successful connexion with the periphery. Embryological studies have shown that the periphery provides the conditions necessary for continued growth and maintenance of neurones in the early stages following the first outgrowth of axons (Hamburger & Levi-Montalcini, 1949; Hughes & Tschumi, 1958; see Hughes, 1968). However, little is known about the factors which influence the growth of axons at later stages, when their peripheral connexions have been established. Corner & Schadé (1967), reviewing the factors which are known to influence the maturation of nerve cells, included a possible role for electrical activity, and indeed a few previous histological studies of the developing peripheral nervous system in mammals have suggested that the amount of activity an axon undergoes may in some way influence its final size (Aitken, Sharman & Young, 1947; Evans & Visoso, 1951; Tomanek & Tipton, 1967). Fernand & Young (1951), in an anatomical study of the sizes of muscle nerves in the adult rabbit,

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suggested that some 'influence' received by the neurone from afferent and central sources supplemented a general morphogenetic factor in determining the sizes of nerve cells and fibres.

The object of the present series of experiments was to assess electrophysiologically the importance of activity in the developing peripheral nervous system of the rat. The rat is born with an immature nervous system relative to the new-born of most mammals and is therefore ideally suited for experimental manipulation at early stages of development. Various techniques are available for producing degrees of inactivity in the otherwise intact neuromuscular system. Limb immobilization (Fischbach & Robbins, 1969), spinal cord isolation (Tower, 1937), chemical agents which interfere with neuromuscular transmission or impulse conduction (Drachman, 1968) and tenotomy (Eccles, 1944) have all been widely used in several species. However, tenotomy of the intertransverse caudal muscles of the rat tail was chosen as the principal lesion in the experiments to be reported here since it provided a simple and relatively efficient way of reducing the usage of nerve and muscle from the earliest post-natal stages of development. A preliminary account of this work has been published (Gladden & Russell, 1975).

METHODS

The intertransverse caudal muscles of the rat lie deep at the base of the tail and consist of six to eight individual slips of muscle. The muscle takes origin from the sides of the vertebrae at the base of the tail, and each slip of muscle has a long tendon which runs the length of the tail and inserts into the sides of the more distal caudal vertebral bodies. In each experiment only the innervation of the last (most distal) slip of the intertransverse caudal muscle was studied. The normal last slip of the intertransverse caudal muscle has four to six motor units receiving their innervation from a small branch of the dorsal collector nerve trunk. The sensory innervation of the last slip of the rat intertransverse caudal muscle has been studied by Gladden (1969) and found to consist of one or two muscle spindles and occasionally one or two Golgi tendon organs. The muscle has a more widely variable number of group III afferents, ranging from none to five. Axons from the last slip lie solely in the third caudal dorsal and ventral roots, while axons from the more proximal slips run in the second and first caudal roots. This facilitates isolation of the afferent and efferent nerves to this particular slip of muscle.

Tenotomy

Bilateral tenotomy of the last few slips of the intertransverse caudal muscle was simply carried out by removal of the last two or three caudal vertebrae, and in this way freeing the tendons from their insertions. In the new-born animal this was done by docking about 3 mm from the end of the tail. Bleeding is minimal, and the operation can be carried out quickly and simply under aseptic conditions.

From any one litter half the males were tentomized at birth in this manner and the other half served as controls. After tenotomy, the animals were left for a period of 8 weeks alongside their litter-mates before further experiments were carried out. Those 8 weeks encompass the period of greatest rate of nerve maturation in the rat (Friede & Samorajski, 1968). Hereafter, unless specifically referred to as adult, all tenotomized preparations will therefore have been tenotomized at birth, and, along with controls, kept until the age of 8 weeks. The overall growth of the tail in tenotomized preparations during this period was not seriously affected. The tails of those animals were 4–5 mm shorter than normal and did not taper to a point as in normal animals.

In this preparation there is no simple means of obstructing the course of readhesion of the cut tendon to the remaining caudal vertebrae. Reattachment of the tendon is likely to occur at some point within the 8-week experimental period. However, it is most likely that the muscle remains effectively tenotomized for a significant proportion of the 8-week period (see Discussion).

Tenotomy of the intertransverse caudal muscles of adult rats was carried out under ether anaesthesia, by making a small incision in the skin of the tail about two thirds along its length thus exposing the bundle of tendons running from the intertransverse caudal muscle to the distal caudal vertebrae. The bundle of tendons was picked up and a 3 mm length of tendon excised from each. These adult animals in which the intertransverse caudal muscle had been tenotomized were also kept for an 8-week period before further experimentation.

Deafferentation

The second and third caudal dorsal root ganglia were surgically removed under ether anaesthesia in normal 2-week-old male rats. It was not possible to operate successfully on animals younger than 2 weeks. The dorsal root ganglia were exposed by cutting away the top surface of the sacro-caudal vertebrae, which had not yet calcified. The ganglia can be seen under high magnification as small semi-opaque swellings in the roots. Care was taken to minimize damage to the underlying ventral roots. The incision was sutured, and in the ensuing weeks regularly sprayed with antibiotic (Polybactrin, Calmic Medical Ltd.). By 8 weeks of age the animals which survived showed little sign of the earlier surgery. The extent of deafferentation was checked electrophysiologically during the subsequent measurement of motor nerve conduction velocities.

Measurement of conduction velocities

The male albino rats, whether normal controls or in which the intertransverse caudal muscles had been either tenotomized or deafferentated, were anaesthetized with urethane (170 mg/100 g body wt.) injected intraperitoneally on reaching approximately 8 weeks of age. The age of animals used at this stage ranged from 54 to 62 days (mean 57·8 days) and weights ranged from 150 to 230 g (mean 188 g). The animals were then set up for spinal root recording using the preparation described elsewhere in detail by Gladden & Kidd (1969). Basically, this consists of exposing the caudal spinal roots and the terminal slips of the intertransverse caudal muscle and forming a small pool at both of these sites.

The conduction velocities of motor and sensory axons innervating the last slip of the intertransverse caudal muscles were measured by stimulating the muscle nerve branch close to its entry into the last slip and recording in the third caudal dorsal or ventral root. Conduction distances ranged from 55 to 63 mm. Since the nerve branch to the last slip contains relatively few axons, the conduction velocities of individual axons could be accurately measured by slowly recruiting them in the compound action potential recorded in the whole spinal root. The stimuli to the nerve branch (0·1 m/sec width, 1/sec) were localized to this small region by using bipolar silver wire (0·01 in. diam.) electrodes insulated except at the tip with a fine coating of Araldite (Ciba) resin. The conduction velocity of individually recruited nerve axons was calculated from the latency between the stimulus artifact and the beginning of the rise of the corresponding wave of the compound action potential, and the conduction distance measured at the end of the experiment. In all cases latencies of single action potentials were, however, measured at the threshold stimulus strength required to recruit them, to prevent stimulus escape and excitation of the axon at a distance from the cathode (Kidd, 1968). Several oscilloscope sweeps of each recruited potential were checked to ensure that, at threshold stimulus values, the latency of the unit did not alter. Myoshi & Goto (1973) report that the conduction velocity of the fastest conducting motor axons to the distal segmental muscles of the rat tail increases linearly with temperature at a rate of about 1·3 m/sec per degree between 24 and 42 °C. It was therefore essential to maintain the animal's rectal temperature with heat lamps within the narrow range 35–37 °C. Along approximately 80% of the conduction distance the nerve is unexposed, but the temperature of the exposed lengths of axon in the tail and spinal pools, which are an integral part of the basic preparation, were monitored with bead thermistors and maintained close to 37 °C by regularly exchanging the Krebs solution and paraffin in these pools.

With these precautions the accuracy of conduction velocity measurements in different experiments was standardized. The largest inherent source of error in any one experiment was the measurement of conduction distance. This was minimized in each case by dissecting the tissues surrounding the nerve trunk between the tail and spinal pools such that a thread could be laid along the route of the nerve to give a measure of the interelectrode distance.

RESULTS

Conduction velocity of afferent axons in control animals

Fig. 1*A* shows the distribution of the conduction velocities of afferent axons in the nerve branch supplying the last slip of normal intertransverse caudal muscles of

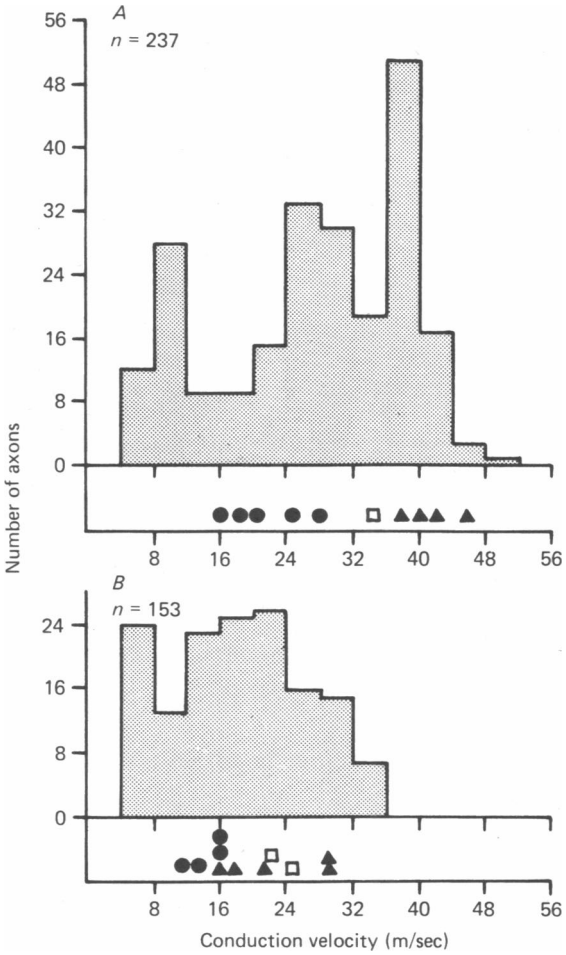

Fig. 1. The distribution of conduction velocities of sensory axons from the last slip of intertransversal caudal muscles of 8-week-old male rats. Sensory axons supplying normal muscle (*A*) and muscle chronically tenotomized at birth (*B*). The symbols on the lower horizontal axis in each case represent the position within the conduction velocity spectrum of individually identified axons from muscle spindle primary endings (▲), muscle spindle secondary endings (●), and tendon organs (□).

thirty-two 8-week-old rats. These axons exhibit a classically trimodal distribution, with peaks at 38, 26 and 10 m/sec. This corresponds to a division into the categories of group I, group II and group III previously described for muscle nerves. The slowest conducting group (group III, 4–16 m/sec) comprise about 21 % of the total sample. Group I and group II populations will certainly overlap (group I, 32–52 m/

sec; group II, 16–36 m/sec) and no simple division can be made between these two peaks of the histogram.

It is now well established for the cat hind limb (Boyd & Davey, 1968) that in a pure muscle nerve like this one the majority of group I axons originate in muscle spindle primary endings and Golgi tendon organs. Similarly, axons in group II originate principally in muscle spindle secondary sensory endings. However, this division of the histogram into functionally distinct populations has not been studied to the same extent in the rat. Andrew, Leslie & Thompson (1973) have shown that although for the muscles of the rat leg the familiar situation prevails as in the cat, the distal *segmental* muscles of the rat tail can have spindle primary and secondary afferent axons both with conduction velocities at the fast end of the range. In the present study the recognition of the function of single unit afferent filaments in the dorsal roots was based on their behaviour during a ramp and hold stretch applied to the muscle slip, and during muscle twitches initiated by stimulating the third caudal ventral root (B. H. C. Matthews, 1933; see P. B. C. Matthews, 1972). The individual shapes on the lower axis of Fig. 1*A* represent the position within the conduction velocity spectrum of axons whose function had been identified. Four afferents from muscle spindle primary sensory endings (group Ia) in the last slip of the normal intertransverse caudal muscle were found within the range 36–48 m/sec. One group Ib afferent from a Golgi tendon organ had a conduction velocity of 35·0 m/sec, just slower than the observed range for spindle primary afferents. Almost certainly, however, if larger numbers of single units had been firmly identified there would be some overlap between the observed groups Ia and Ib conduction velocity ranges. The five units identified as originating in spindle secondary endings of normal muscles were found in the range 16–32 m/sec. These results confirm that, for afferent axons from the normal intertransverse caudal muscle of the rat, the pattern of distribution within the conduction velocity spectrum is the same as has been previously established for the cat hind limb.

Conduction velocity of afferent axons after muscle tenotomy at birth

The effect of tenotomy of the terminal slips of the intertransverse caudal muscle at birth on the conduction velocity attained by the afferent axons from the last slip of the intertransverse caudal muscle is shown in Fig. 1*B*. The histogram is compiled from the results from twenty-four 8-week-old rats. The last slip of the tenotomized intertransverse caudal muscle at 8 weeks of age showed no gross signs of atrophy, but muscle fibre cross-sectional area measurements revealed that the tenotomized muscle fibres were significantly smaller than their normal counterparts (Russell, 1977*a*). The conduction velocities of the afferent axons are distributed very differently in comparison with the controls (Fig. 1*A*). This redistribution is statistically significant ($P < 0{\cdot}0001$) using the Kolmogorov–Smirnov test for large samples of non-parametric data (Focal 8-276, Decus Program Library; see Massey, 1951). The trough between 12 and 24 m/sec obvious in the normal histogram is replaced by slowly conducting axons comprising 48% of the whole sample. 38% of axons of normal muscle were found in the range 32–52 m/sec, but in tenotomized muscle very few afferent axons (4·5%) were found with conduction velocities greater than 32 m/sec. It is difficult to say whether axons in groups I, II or III have been preferentially

affected by tenotomy of the muscle, although it certainly seems that axons with conduction velocities in the normal group I range are most notably absent, and that a greater proportion of axons are found at the lower end of the group II range.

One of the first questions which this result raises is whether this redistribution of conduction velocities is simply due to a selective loss of the fast-conducting afferents. The normal variation in the extent of the sensory innervation of the last slip of the intertransverse caudal muscle prevents simple comparison of the number of afferents from normal and tenotomized muscles. It has been shown, however, that muscle spindles and tendon organs survive muscle tentotomy during the growth period in rats (Zelena, 1962; Gladden, 1971), but it was necessary in the present study to determine whether the innervation of these sensory receptors remained functionally intact.

The individual shapes on the lower axis of Fig. 1*B* show the position within the conduction velocity spectrum of eleven afferent axons of identified origin, each from a separate animal. Examples of tendon organ, spindle primary, and spindle secondary ending afferents were all found, and were grouped close together in the range 12–32 m/sec. Tendon organ and spindle primary ending axons still have conduction velocities at the faster end of this new range. However, the magnitude of the change in conduction velocity for these group I afferents appears to be greater than for group II afferents. In both normal and tenotomized cases no axons were found responding to stretch in the range 4–12 m/sec. This range presumably can be attributed to group III myelinated afferents, which were not specifically identified in these experiments, but may be largely unaffected by tenotomy.

Conduction velocity of efferent axons in control animals

Fig. 2*A* shows the distribution of conduction velocities of efferent axons supplying the last slip of the intertransverse caudal muscle in forty-seven male rats at 8 weeks of age. The distribution is markedly bimodal with the faster group conducting in the range 24–52 m/sec, and the slower group in the range 4–24 m/sec. This corresponds to a division into extrafusal (α) and intrafusal (γ) motor innervation described for the rat soleus muscle (Andrew & Part, 1972) and for the rat caudal segmental muscles (Andrew & Part, 1974). The 'shoulder' on the α peak between 24 and 36 m/sec corresponds to the range in which Andrew & Part (1974) found motor axons supplying only slow-twitch motor units in the segmental tail muscles. In the present study the smallness of this proportion of the population is presumably related to the fact that only 15% of all the muscle fibres in the last slip of the intertransverse caudal muscle were histochemically identified as being of the slow-twitch type (Russell, 1977*b*).

Conduction velocity of efferent axons after muscle tenotomy at birth

The conduction velocities attained by efferent axons supplying the last slip of tenotomized intertransverse caudal muscles during the first 8 weeks of development after birth are shown in Fig. 2*B*. The distribution of conduction velocities is again markedly bimodal, but the faster group of axons now have a peak at 28–32 m/sec, whereas the slow peak remains at around 8–10 m/sec. This redistribution of conduction velocities is statistically significant ($P < 0{\cdot}0001$), and in marked contrast to

normal, very few fast conducting axons were found with conduction velocities greater than 32 m/sec. 63% of the efferent axons supplying normal muscle conduct faster than 32 m/sec, compared to only 10% in tenotomized muscles. The range of the fastest motor axons supplying tenotomized muscle is now limited to 20–44 m/sec, and most of these axons have conduction velocities in the range which was previously attributed to the slow α motor axons of normal muscle.

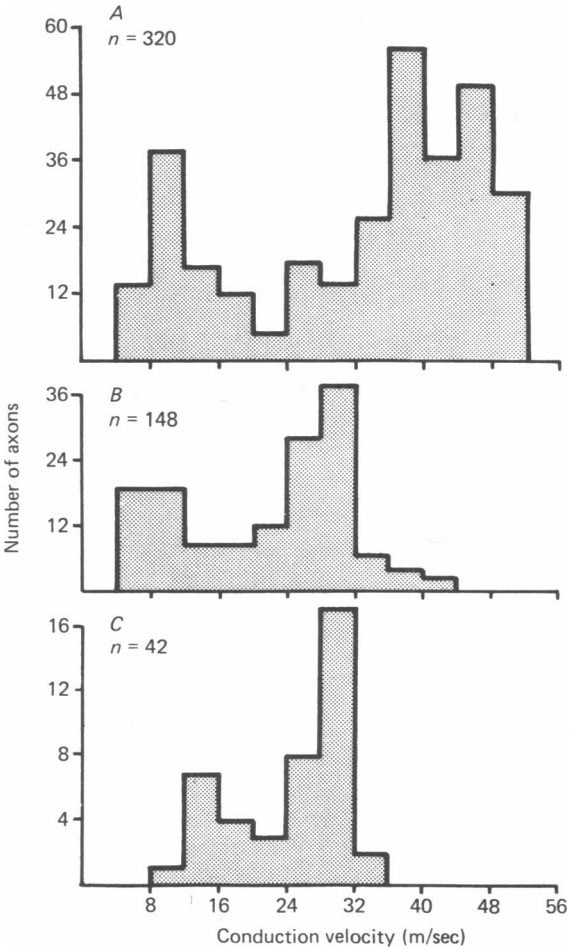

Fig. 2. The distribution of conduction velocities of motor axons supplying the last slip of intertransverse caudal muscles of 8-week-old male rats. Motor axons supplying normal muscles (*A*), muscles chronically tenotomized at birth (*B*), and muscles chronically deafferentated from 2 weeks old (*C*).

Since motor axons with conduction velocities in the normal fastest range are not found supplying the muscles which had been tenotomized at birth this again raises the question whether tenotomy has caused selective degeneration of only these fast α axons. Straightforward comparison of the number of motor units in normal and tenotomized muscles showed that this was not the case. The number of motor units and the over-all dimensions of the normal last slip of the intertransverse caudal muscle do not vary widely. In normal cases, the number of α motor axons varied

between four and six in different animals, with a mean of 4·90. Polyneuronal innervation of muscle fibres is unlikely at 8 weeks in the rat (Redfern, 1970; O'Brien, Purves & Vrbova, 1977) and each α motor axon can be assumed to be supplying a distinct group of muscle fibres. The motor innervation of the tenotomized last slips of the intertransverse caudal muscle was first shown to be intact by stimulating the ventral root and observing the muscle twitch. These motor axons were presumably still among the fastest conducting of all the efferents supplying tenotomized muscle since they had the lowest stimulus threshold.

However, if the fastest α motor axons had degenerated it is possible that the remaining motor axons might have branched to reinnervate the deprived muscle fibres, but a smaller number of motor units would have been observed in the compound action potential. This was not the case since the number of extrafusal motor axons to the tenotomized last slips of all the intertransverse caudal muscles varied from four to six, with a mean of 4·95. Thus tenotomized muscle is supplied by the normal number of extrafusal motor axons which have conduction velocities significantly slower than normal.

Conduction velocity of axons supplying tenotomized adult muscles

The terminal slips of the intertransverse caudal muscles of twelve adult rats (body weight > 400 g) were tenotomized and left for a period of 8 weeks. These muscles were therefore tenotomized for the same period as in the young animals. Normal adults of similar weights served as controls. On dissection after 8 weeks the adult tenotomized muscles were markedly atrophic. Fig. 3*A* compares the distribution of conduction velocities of normal and tenotomized efferent axons. Both histograms show the expected bimodal distribution of efferent conduction velocities corresponding to α and γ populations. Most importantly, efferent axons to tenotomized adult muscles were found with conduction velocities as fast as those to normal muscle. Similarly, in Fig. 3*B* afferents from tenotomized adult muscle were found with conduction velocities as fast as those from normal muscle and, as with the efferents, the distribution of conduction velocities was not significantly altered by tenotomy (Kolmogorov–Smirnov test). It is therefore apparent that the effect of tenotomy on the conduction velocities of the innervation of the intertransverse caudal muscle is limited to growing animals. This is in keeping with the results of previous histological and electrophysiological studies which have found no significant changes in axon size or conduction velocity as a result of tenotomy of adult muscles (see Discussion).

The effect of deafferentation during development on motor nerve conduction velocities

If the principal effect on tenotomy is to impose 'disuse' or 'reduced usage' on the muscle and its innervation then useful comparison may be made with the effect of removal of the homonymous afferent input to the motoneurones supplying the last slip of the intertransverse caudal muscle. The terminal slips of the intertransverse caudal muscles of normal young animals were deafferentated at 2 weeks of age, and these animals were kept until the age of 8 weeks to allow the results to be compared to the control and tenotomized groups. The results from six successfully deafferentated 8-week-old animals are shown in Fig. 2*C*.

The conduction velocities attained by motor nerves to deafferentated muscle exhibit a bimodal distribution with peaks at 14 and at 30 m/sec. No axons were found with conduction velocities greater than 36 m/sec. This distribution of conduction velocities looks essentially similar to that for the efferents to tenotomized muscle, with the relatively fast, low threshold axons being found only in the range 20–36 m/sec. Indeed it is not statistically significantly different from Fig. 2*B*. The average number of extrafusal motor axons innervating the deafferentated muscle was five, within the range for normal muscle of four to six. The lack of fast conduction velocities cannot therefore be due to preferential loss of the largest motor axons, and for the same reason cannot be the result of damage to the ventral roots during the removal of the dorsal root ganglia. At the time of the final experiment

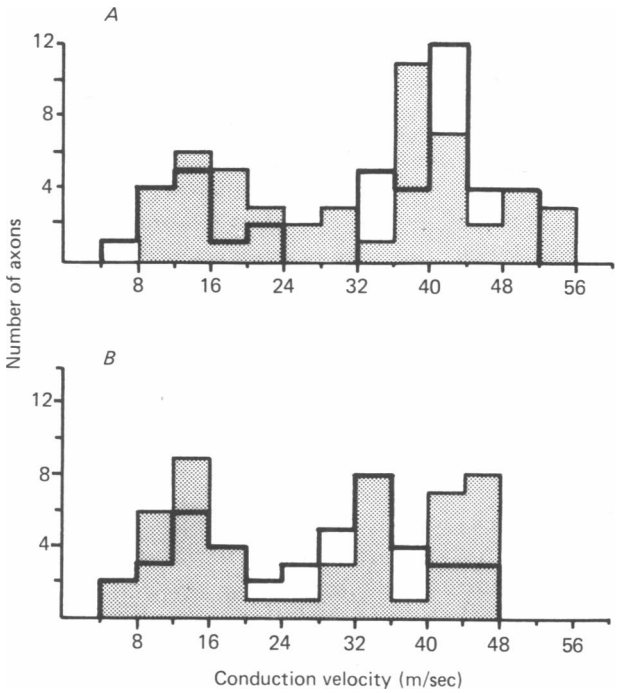

Fig. 3. The distribution of conduction velocities of axons supplying the last slip of intertransverse caudal muscles of adult rats. Sensory axons from normal adult muscles (*A*), and tenotomized adult muscles (*A*, stippled). Motor axons supplying normal adult muscles (*B*), and tenotomized adult muscles (*B*, stippled).

the muscle nerve branch to the last slip of the intertransverse caudal muscle was stimulated, and recording electrodes placed on the third, second and first caudal dorsal roots in succession to check the extent of deafferentation. In all deafferentated animals no activity could be recorded in any of the caudal dorsal roots in response to this stimulus, showing that deafferentation of the last slip of the intertransverse caudal muscle had been complete.

DISCUSSION

The results show that tenotomy during development causes significant changes in the conduction velocities of both sensory and motor axons supplying the muscle. In particular, it seems that the axons which would normally have been destined to be among the fastest conducting have considerably slower conduction velocities than their normal counterparts. It is also evident that this effect of tenotomy is limited to growing animals, since tenotomy in the adult caused no slowing of conduction velocities; a result in agreement with previous electrophysiological and histological studies on the hind-limb muscles of the adults of different species. Beránek, Hník, Vyklický & Zelená (1961) showed that chronic tenotomy in adult cats produced no difference in the diameter or conduction velocity of nerve fibres. Aitken *et al.* (1947) showed that Achilles tenotomy in adult rabbits caused no significant change in nerve fibre diameters 100 days later. Hník Beránek, Vyklický & Zelená (1963) reported insignificant changes in number and diameter of nerve fibres supplying tenotomized adult cat muscles even 20 months after tenotomy.

The results of the present study therefore suggest that tenotomy during development retards the increases in axonal dimensions which would normally occur during this period in the rat. This assumes, however, that conduction velocity can be regarded as a reliable index of axon calibre and neuronal cell body size during development. There is substantial evidence in the literature to support this assumption (Hursh, 1939; Friede & Samorajski, 1968; Martinez & Friede, 1970). A corollary of this conclusion is that, once final dimensions have been reached in the adult, the spectrum of axonal conduction velocities found in the adult animal should reflect differences in the size of the neurones from which they arise. Kernell (1965), Barrett & Crill (1971, 1974) and Burke (1973) have demonstrated with intracellular recording techniques that axonal conduction velocity and motoneurone input resistance are indeed correlated with the anatomical size of motoneurones. On the basis of this argument it was considered that conduction velocity measurements could indicate reliably the extent of neuronal growth during the post-natal period. Histological analysis, on the other hand, would have posed many interpretative difficulties due to the artifacts arising from the variable extent of fixation shrinkage, the necessity for precise transverse planes of section in axon measurement, and the difficulty in selecting appropriate planes of section if neurone cell body dimensions were to be measured. Changes in internodal distance are unlikely to have been responsible for the observed changes in conduction velocity, since Sanders & Whitteridge (1946) found that after full regeneration nerves conducted at normal velocity when their diameter and myelin thickness had returned to normal although the internodal distance was halved. Cragg & Thomas (1957) have also shown that during development of the lateral line nerve of the trout, conduction velocity is not directly related to internodal distance but follows the increase in total diameter of the nerve fibres.

Previous histological studies of the innervation of muscles tenotomized during development have shown only small changes in nerve total diameter. Aitken *et al.* (1947) found that nerves which had regenerated into tenotomized rabbit muscle were significantly smaller when compared to controls regenerating into normal muscle. This prompted them to suggest that effective functioning was necessary for matur-

ation of regenerating nerve fibres. Evans & Viososo (1951) studied this effect under conditions of normal development and found there was a small but statistically significant decrease in the total diameter of the largest group of fibres in mixed muscle nerves innervating tenotomized muscle. They concluded that since there was little retardation of growth of the nerves, activity plays but little part in determining nerve fibre size. They did, however, recognize that the magnitude of the effect may depend on the growth period under investigation, and in fact their tenotomies were performed at 3 weeks in the rabbit and left until the age of 16 weeks. This may be too late for the lesion to have a large effect. Similarly, Tomanek & Tipton (1967) saw only a very small reduction in total diameter of all nerve fibres to rat hind-limb muscles which had been tenotomized at 10 weeks of age and left for a further 8 weeks. It is now probable that larger effects on nerve fibre total diameter would have been observed had these tenotomies been performed at the earliest possible age.

The use of tenotomy as a means of reducing usage of a muscle and its nerve is generally considered to be fraught with interpretative uncertainties. Estavillo, Yellin, Sasaki & Eldred (1973) have, however, provided the most recent review of the situation with respect to hind-limb muscles and the weight of the evidence in the literature supports the view that tenotomy must limit, but not necessarily completely silence, impulse activity in the sensory and motor nerve supply. Thompson (1972) mentions that the impulse traffic in the caudal dorsal roots containing afferents from the intertransverse caudal muscles is readily silenced by cutting the intertransverse caudal muscle tendons and allowing them to retract. Acute tenotomy also causes immediate silencing of the intertransverse caudal muscle electromyogram (N. J. W. Russell, unpublished observations). In the rat soleus Karpati, Carpenter & Eisen (1972) found that Achilles tenotomy reduced the aggregate electromyographic activity during any given recording period to about 60% of normal with long periods of electrical silence interspersed.

Reattachment of the cut tendon is an inherent problem when tenotomy is used as an experimental lesion. Previous workers have used a variety of methods to prevent or delay reattachment of the tendon, usually the readily accessible hind-limb tendons of adult animals, and precautions of this nature could not be applied to tenotomy of the intertransverse caudal muscle of new-born rats. Although Buller & Lewis (1965) reported that the readhesions of the adult rabbit Achilles tendon observed 17–85 days after tenotomy were broken down by mild pressure and appeared not to be functionally significant, a sample of four chronically tenotomized intertransverse caudal muscles from the present series of experiments showed phasic electromyographic activity at 8 weeks of age which was indistinguishable from normal. However, in none of the 8-week-old rats with tenotomized muscles were the axonal conduction velocities found to be distributed normally, and this consistency suggests that if any possible premature tendon reattachment could have been avoided then the effect would have been even greater.

The effect of deafferentation on the axonal conduction velocity attained by motoneurones in the caudal spinal cord during development is essentially similar to tenotomy. The effect which both of these lesions have in common is the imposition of a reduction in the afferent input to the developing motoneurones which supply the intertransverse caudal muscle. Indeed, deafferentation is also similar to tenotomy

in that the effect appears to be limited to growing animals. The conduction velocities and diameters of motor nerves in the adult cat hind-limb are unaffected by de-afferentation (Boyd & Davey, 1968; Kuno, Miyata & Munoz-Martinez, 1974).

The results of the present study therefore suggest that neurones in the peripheral nervous system may exhibit a dependence on activity to complete their growth, and thus complement the intrinsic genetic determinants of neuronal development. There is substantial evidence in the literature which suggests that this hypothesis is equally applicable to other regions of the nervous system. For example, cells in the lateral geniculate nucleus were smaller when deprived of activity during development (Wiesel & Hubel, 1963; Hubel & Wiesel, 1970; Hickey, Spear & Kratz, 1977), and in the kitten spinal cord, Smith (1974) has shown that neurones of Clarke's nucleus, deafferentated by dorsal root section at 3 days after birth, were markedly smaller than controls. The results of Lewis, Bagust, Webb, Westerman & Finol (1977) apparently demonstrate a change in conduction velocity of axons brought about by changes of activity in adult animals. However, in those cross and self-reinnervation experiments the motoneurones are regenerating, which in terms of nerve growth is presumably a similar situation to normal development. The morphological effects of sensory deprivation in the central nervous system have most recently been reviewed by Globus (1975) and in all cases the effects of reduced activity are found to be much less pronounced, if not absent, in the adult animal.

Thus it is clear that neuronal activity can influence neuronal growth, and while accepting the limitations of tenotomy as a technique for imposing disuse, it is reasonable to suggest from the present results that activity is a prerequisite for normal neuronal calibre increases during development of the peripheral nervous system. It may be envisaged therefore that the mature nervous system must be the product of two distinct mechanisms. At the earliest stage rigid intrinsic genetic control would establish the gross morphogenesis of a system which could thereafter interact with its environment. By means of the activity in the nervous system which this interaction produces, the various elements would be able to respond in appropriate directions during development such that the optimum functional properties of the system may be attained by the time the animal reaches maturity.

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